

reaction. In Galim the annual transmission of *O. volvulus* was high (2,500 L3/man) and would normally cause hyper-endemic onchocerciasis. However, the mean microfilarial density in the whole population was strikingly low (0.034/snip) and 107 (!) times lower than in Karna (36.29), where the *O. volvulus*-transmission was 12 times lower (Figure 1). This could possibly be due to the very high transmission of *O. ochengi* in Galim (33,000 L3/man x year) which might induce a partial crossreactive immunity in the local population. This hypothesis is strengthened by first immunological studies which demonstrated a high degree of homology between *O. volvulus* and *O. ochengi* in protein profile and serological recognition, and showed significant differences in the serological reactivity between patients from Galim and Karna (Hoch *et al.*, 1992). It is thus concluded that the proximity, throughout the year, of large numbers of cattle infected with *O. ochengi* probably protect the local human population from severe onchocerciasis

ACKNOWLEDGEMENTS

The technical assistance of M. D. Ekale is gratefully acknowledged. This investigation received financial support from the Commission of European Communities (TS2/0184-D (AM) and TS3/CT92-006).

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PREPATENCY PERIOD AND SOME ASPECTS OF THE EPIZOOTIOLOGY OF *ONCHOCERCA OCHENGI* INFESTATION IN CATTLE IN THE ADAMAWA PLATEAU, CAMEROON

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KEY WORDS : *Onchocerca ochengi*. epizootiology. prepatency. infective larvae. cattle. Cameroon.

SUMMARY

In an onchocerciasis endemic area, calves which were one to 24 months old, were examined for palpable *Onchocerca ochengi* nodules and microfilariae in skin snips. A highly infested bait oxen was used for the production of infective larvae through the vector *Simulium squamosum*. A prepatent period of about 10 months for the appearance of palpable nodules and skin microfilariae was identified, and the prevalence of nodules (80.5 %) and the microfilarial density (0.74 microfilariae per mg skin biopsy) was highest in the 19-24 months old animals. Peaks of 4.8 infective larvae per blood fed fly were reached during infective larvae production. The ease of counting palpable nodules, collecting blood and skin snips, performing nodulectomy, isolating adult worms, keeping these *in vitro* and producing infective larvae provides favorable conditions for the use of this animal model for *in vivo* chemotherapy and vaccine development research.

INTRODUCTION

Although there is a high prevalence of bovine onchocercosis in North Cameroon coupled with a high incidence of multiple-species concurrent infestations (Wahl *et al.*, in press), very little knowledge exists on the biology, immunology and pathology of *Onchocerca ochengi* in particular. There is emphasis on the study of this filaria because its life cycle, infective larvae (Wahl *et al.*, 1991) and nodule formation are very similar to those of *O. volvulus*. The need for expanded research on most of these aspects of bovine onchocercosis has been growing in recent years because of its possible implication in the control (zooprophylaxis and crossreactive concomitant immunity) of human onchocerciasis in a highly endemic region (Renz *et al.*, 1989 ; Wahl, 1991 ; Hoch *et al.*, 1993).

In this survey we investigated the rate of acquisition of palpable nodules and skin microfilariae of *O. ochengi* during natural infestation in cattle with a view to find out the prepatent period of the parasitism. Another objective was to find out how feasible it was to use cattle naturally infested with *O. ochengi* to produce infective larvae which were needed for immunological studies.

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MATERIALS AND METHODS

NODULE FORMATION AND PREPATENCY OF *O. OCHENGI*

17 calves of Zebu cattle (Gudali breed) of the age between one and 24 months were examined in January 1993 for *O. ochengi* nodules and skin-microfilariae load in the Dawa ranch about 15 km from Ngaoundere and very close to the river Vina du Sud, where the Annual Transmission Potential of *O. ochengi* larvae was estimated to about 60,000 L3 per cattle per year (Wahl, 1991). After aging the animals by dentition to the nearest month and/or branded ages by the ranch owner, they were cast to ground and restrained with ropes. All palpable nodules of *O. ochengi* were counted by inspecting both sides of the ventral skin of the animal. From the shaved skin, three 'skin-biopsies' were taken with a scalpel from along the linea alba, one just posterior to the umbilicus, one mid way between umbilicus and udder and one closely anterior to the udder/scrotum. The skin samples were weighed (average weight 60 mg) and incubated in one ml RPMI 1640 medium (complemented with penicillin and streptomycin) at 37° C for 24 hrs. Then the microfilariae were identified to their species and counted (microfilariae per mg, mff/mg).

PRODUCTION OF INFECTIVE LARVAE (L3) OF *O. OCHENGI*.

A bull highly infected with *O. ochengi* (49 palpable nodules and 10.6 mff/mg of skin tissue without digestion with collagenase and 13.1 mff/mg after digestion) was used as a bait animal for catching *Simulium squamosum* flies at the river Vina du Sud near Wakwa. Flies coming to feed on the animal (mainly around the scrotum and penis) were caught in polystyrene tubes when they had completed their blood-meal. They were kept in dark incubators at 22-24° C, 80 % humidity and fed on cotton balls containing 30 % sugar solution (supplemented with Nipagin fungicide). Flies which died within the first two days were dissected to evaluate the natural infection rate in wild caught flies. After 9 days, the surviving flies were dissected in RPMI medium, and all L3 emerging from the head, thorax and abdomen were collected for subsequent inoculation of a calf and four rodent species (Wanji *et al.*, in prep.) or for obtaining excretory/secretory products.

RESULTS AND DISCUSSION

PREPATENCY PERIOD OF *O. OCHENGI*

one of 9 calves younger than 10 months had nodules or microfilariae. The first palpable nodules were detected in 2 of 15 calves examined in the age of 10 months (1 and 3 nodules palpated), but only the animals with 3 nodules also showed microfilariae (0.024 mff/mg). At the age of 11 months, 5 of 17 calves showed nodules and 5 of 6 animals at the age of 12 months. One animal also had microfilariae. The following increase in the appearance of nodules and microfilariae was very dramatic, 3.3 nodules

per animal within 12 months, reaching a peak infestation in the oldest animals (Table I). Most *O. ochengi* nodules were seen on the ventro-posterior aspects of the animal with a tendency to concentrate on the udder and scrotal regions.

The age related increase in *O. ochengi* infestation in calves and weaners which we observed here completed data on the prevalence of nodules and microfilariae in adult cattle reported before in the same area by Trees *et al.* (1992) and Wahl *et al.* (in press). These authors concluded that the decline in microfilarial load they observed in the oldest age-group (> 8 years) possibly indicated acquired resistance in the high transmission area.

The present data indicate that the development of infective L3 to the adult stage (palpable nodule) takes less than 10 months and microfilariae in the skin can be detected from 10 months onward. The very few microfilariae which might be found in the foetus or new born calf (Daiber, unpublished data), most probably stem from adult worms in the mother. This prepatency period of about one year coincides with the annual variations of the *Simulium* vector biting rate, and it is tempting to speculate that the seasonal fluctuation in the microfilarial density in cattle (unpublished data, and below) reflects this prepatency of microfilarial production which is then followed by an immune-mediated downregulation of the microfilarial load in the skin.

Age (months)	1-6	7-12	13-18	19-24
n/117	5	41	30	41
Total no. of nodules	0	26	71	158
Mean of nodules	0.0	0.6	2.4	3.9
SD ± of nodules	0.0	1.2	3.5	4.7
Mean MfD*	0.0	0.07	0.115	0.736
% Mf+ ()	(0.0)	(0.05)	(36.7)	(56.1)
% Nodule+	0.0	29.3	70.0	80.5

Table I. – On-set and age distribution pattern of *O. ochengi* palpable nodules and microfilariae in cattle of the Adamawa Plateau. All examinations for nodules done in Jan. 1993.

MfD = Microfilariae density, (.) = % of animals positive for Mf.

First animal with nodule seen at age of 10 months.

First animal with microfilariae was 10 months old.

* without digestion of the skin-biopsy.

PRODUCTION OF INFECTIVE LARVAE (L3) OF *O. OCHENGI* IN *S. SQUAMOSUM*

A total of 2,135 bloodfed flies were collected from the bait oxen, of which 44.5 % survived until day 9, when the microfilariae had completed the development to infective L3. Peak L3 production rates of 4.8 larvae per surviving infected fly were attained in the dry season (range 1.0 to 4.8 during 18 experiments) but the maximum in the rainy season was 0.7 (range 0.1 to 0.7 during 11 experiments, Table II). There was also an apparent decrease of natural infestation rates of *S. squamosum* at the study site at the

onset of the rainy season, and coincidentally, the microfilarial density in the skin of the bait animal dropped by at least 10 fold (Table II). Flies dissected within 48 hrs after a bloodmeal on the oxen had ingested 8.5 mff during the dry season and 0.6 in the rainy season.

This decline in skin microfilarial density and uptake by feeding *S. squamosum* flies could be attributed to the above mentioned seasonal changes in the intensity of natural transmission or in microfilarial production and turnover, but it is more likely that it was due to an acaricide treatment (Alphacypermethrin) of the animal on the 20th March 1993, which became necessary because of its high tick infestation at the onset of the rainy season.

	DRY SEASON*	RAINY SEASON
Wild infection L1, L2, L3 per fly dissected (number flies dissected)	1.5 (144)	0.6 (239)
Microfilarial density in the skin of the bait animal mff/mg (proportion emer- ged**)	13.1 (80.7 %)	2.2 (53.4 %)
Microfilarial uptake mff/bloodmeal (number flies dissected)	8.4 (447)	0.6 (108)
Proportion of bloodfed flies surviving to day 9 (number flies bloodfed)	43.1 % (1492)	47.2 % (810)
L3 per fly, 9 days after the bloodmeal (number flies dissected)	2.4 (643)	0.3 (382)

Table II. - Variability of natural and experimental infection rates of *Simulium squamosum* with *Onchocerca ochengi* at the river Vina du Sud during the dry and rainy season.

*Dry season : February to 19th of March 1993 (the bait oxen was treated on 20.3.1993). Rainy season : end of March and April 1993

** The skin biopsies were incubated for 24 hours in RPMI medium (proportion emerged) and then digested by collagenase to assess the microfilarial density (microfilariae per mg).

The mortality of flies during the maintenance in the incubator was high, in particular during the first 48 hrs, possibly because of the age of the wild-caught flies (parous rates of about 80 %), the ingestion of high numbers of microfilariae (most of which were trapped in the peritrophic membrane) or inadequate maintenance conditions, such as poor air circulation, very high or low temperatures or excessive humidity. When these conditions are controlled, we expect to achieve a survival rate of about 65 % of bloodfed flies.

The ease of counting palpable nodules, collecting blood and skin snips from *O. ochengi* infested cattle, performing nodulectomy, isolating adult worms, keeping these *in vitro* for excretory/secretory products, and producing infective

larvae present these filarial parasites as a useful model for *O. volvulus* in man, in particular as concerns chemotherapy and vaccine development. Furthermore, the implication of bovine onchocercosis on cattle health, fertility or meat production may be significant and needs further investigation. In cattle herds kept under the same husbandry protocols, some besides the river Vina and others kept on the plateau at Wakwa, it was observed that those along the Vina showed lower calving rates (Mdah and Tawah, unpublished data). The area along the Vina river is now known to be a high perennial transmission zone for *O. ochengi* while the plateau is exposed to very little transmission. In human onchocerciasis, a general and unspecific suppression of cellular and humoral immune response has also been described in patients vaccinated against tuberculosis, rubella or tetanus (Prost *et al.*, 1983 ; Greene *et al.*, 1983). It is thus possible that filarial parasites in cattle could interfere with immune responses to routine vaccination programmes against endemic diseases.

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